FORM-PTO-1390 (Rev. 10-96)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 020755-016

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/402357

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR98/00678	03 April 1998	04 April 1997

TITLE OF INVENTION: TITRATION METHOD FOR A COMPLEX VIRAL COMPOSITION

APPLICANT(S) FOR DO/EO/US: Catherine GERDIL; Jean-Francois SALUZZO

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

420 Rec'd PCT/PTO 04 OCT 1999

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/402357	PCT/FR98/00678	020755-016

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $840.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $670.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $760.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $970.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	6 -20 =	0	X$18.00	$ --	
Independent Claims	1 -3 =	0	X$78.00	$ --	
Multiple dependent claim(s) (if applicable)			+$260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 840.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	-
SUBTOTAL =				$ 840.00	
Processing fee of $130.00for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 840.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 840.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Norman H. Stepno
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404

SIGNATURE

Teresa Stanek Rea
NAME

30,427
REGISTRATION NUMBER

(10/97)

Patent

Attorney's Docket No. 020755-016

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Catherine GERDIL et al	)	Group Art Unit: Unassigned
	)	
Application No.: Unassigned (Corresponds to PCT/FR98/00678)	)	Examiner: Unassigned
	)	
International Filing Date: 03 April 1998	)	
	)	
For: TITRATION METHOD FOR A COMPLEX VIRAL COMPOSITION	)	

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-captioned application as follows:

IN THE CLAIMS:

Kindly amend the claims as follows:

Claim 1, line 4, delete "characterized in that it comprises" and insert --comprising--.

Claim 1, line 8, after "interference," insert --and--.

Claim 2, lines 1-2, delete "characterized in that" and insert --wherein--.

Claim 3, lines 1-2, delete "one of the preceding claims, characterized in that" and insert --claim 1, wherein--.

Claim 4, lines 1-2, delete "one of the preceding claims, characterized in that" and insert --claim 1, wherein--.

Claim 5, lines 1-2, delete "one of Claims 1 to 3, characterized in that" and insert -- claim 1, wherein--.

Claim 6, lines 1-2, delete "one of Claims 1 to 3, characterized in that" and insert -- claim 1, wherein--.

REMARKS

Entry of the foregoing amendments is respectfully requested.

The claims have been amended to eliminate multiple dependency and to place them in better condition for U.S. patent practice.

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____

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Date: October 4, 1999

METHOD OF TITRING A COMPLEX VIRAL COMPOSITION

The invention relates to the field of viral compositions; more specifically the invention relates to a method for determining the virus quantity of each of the species or, more specifically, of each of the serotypes of a given virus species in a composition which contains different species or types of live virus.

Methods for assaying the quantity of each virus species or of each virus type in a viral composition are known in the prior art. The methods which are usually employed consist in using polyclonal antibodies to neutralize the viruses which it is not wished to assay and then in determining the quantity of the remaining virus. This is, in particular, what takes place when there is a need to determine the quantity of attenuated live virus which is present, in the case of each of the I, II and III types, in a composition for vaccinating against polio. However, it is not always possible to implement such methods because it is at times difficult, if not impossible, to neutralize some of the viruses which are present in the composition without interfering with the virus which it is desired to assay, in particular when it is a matter of titrating a vaccine composition which comprises several serotypes of a particular virus, such as the dengue virus, for example. This is because there are no type-specific polyclonal antibodies; monoclonal antibodies which are able to recognize a given type specifically are often not sufficiently neutralizing; sometimes, even, there is no known neutralizing monoclonal antibody. However, in some cases, in particular in the vaccine industry, it is necessary to produce viral compositions which comprise different virus species, or different serotypes of a virus species, in a perfectly defined proportion. Furthermore, pharmaceutical requirements make it necessary to be subsequently able

to quantitatively control the composition of the fabricated products in a reliable manner.

It is therefore desirable to be able to have available methods which enable a complex viral composition to be titrated without being modified.

To this end, the present invention relates to a method for determining the virus quantity of each of the virus types or virus species in a composition containing different species or types of live virus, characterized in that it comprises the following steps:

propagating the viruses of each type or species on cells which are permissive for the viruses but which do not induce any viral interference,

assaying each type or species of the virus using a specific monoclonal antibody.

The method according to the invention is applied to a composition which comprises several different species of virus and/or several serotypes within one and the same species. The viruses can, in particular, be viruses which are responsible for poliomyelitis, rubella, mumps, measles or dengue, or else rotaviruses. The compositions which comprise these viruses can be vaccine compositions in which the viruses, although having an attenuated virulence, are maintained in the live state. The composition can therefore be, for example, a vaccine composition which comprises the three serotypes of the polio virus or a composition which comprises the four serotypes of the dengue virus. The method according to the invention is of particular interest when the viruses are very closely related antigenically and neutralization of one serotype results, by cross-reaction, in the neutralization of the other serotypes.

According to the invention, the viruses which are present in the composition to be tested are propagated, at various dilutions, on cells which are permissive for the viruses but which do not induce any viral interference. It was thus possible to use Vero cells.

The cells are placed in the wells of plates which are suitable for culturing cells and then inoculated with viral suspensions.

The culture medium used for the viral propagation is a conventional medium which is adjusted in accordance with the nature of the cells which are employed and of the virus to be titrated. After incubating for a time which varies depending on the virus (for example a week in the case of the dengue virus), and at the temperature which is optimal for growing the virus under a CO_2 atmosphere, the cell culture supernatants are removed; the cells are then fixed, for example using chilled acetone.

The quantity of viruses present is then determined, for each of the dilutions, using a monoclonal antibody which is specific for the species or the serotype in accordance with the titration performed. The reaction is visualized using a fluorescein-labelled anti-species antibody or using a substrate which is suitable for the ELISA test. The viral titer is determined by the Spearman and Karber method and is expressed as the dose which infects 50% of the cell cultures ($CCID_{50}$).

The same procedure is carried out, in parallel, with each monoclonal antibody which is specific for the species or the type of virus which it is desired to titrate in the viral composition.

It is thus surprisingly possible, using this method, to titrate each serotype which is present in the viral composition without one of the serotypes predominating over the others.

Example

Titration of four monovalent vaccine compositions, each of which comprises a serotype of the dengue virus, and of a composition which has been prepared at the time of assay by mixing equal quantities of the four monovalent compositions tested.

The titration is carried out on 96-well microplates in the following manner:

- consecutive dilutions of each of the compositions are prepared using MEM culture medium which contains 5% foetal calf serum and 2 g/l of sodium bicarbonate,
- the viral suspensions which have thus been obtained are inoculated into Vero cells (ref. ATCC:CCL81VERO), which are in layers which have been established for 1 day, at the rate of 10 wells per dilution. Each valency of the monovalent suspensions and of the tetravalent composition is titrated on at least one 96-well plate,
- the plates are then incubated at 36°C for one week under 5% CO_2,
- the cell culture supernatants are removed and the cells are fixed on the plates using acetone which has been cooled down to -20°C,
- the presence of viruses is detected using a monoclonal antibody which is specific for the serotype which is present in the vaccine composition. The antibodies employed are derived from hybridomas supplied by the CDC (Center of Disease Control, Atlanta, USA).
  The international 1 serotype is labelled with the antibody derived from the hybridoma D2 - 1F1 - 3
  The international 2 serotype is labelled with the antibody derived from the hybridoma 3H5 - 1 - 12
  The international 3 serotype is labelled with the antibody derived from the hybridoma 5D4 - 11 - 24
  The international 4 serotype is labelled with the antibody derived from the hybridoma 1H10 - 6 - 7
- the reaction is visualized using a fluorescein-labelled anti-mouse IgG antibody.

The results are read using a fluorescence microscope. The number of wells exhibiting at least one focus of infected (fluorescent) cells is counted in the case of each dilution.

The titer of the product corresponds to the dilution which results in 50% of the cell sheets (that is 50% of

the wells) being affected and is calculated by the Spearman and Karber method. It is expressed as the $\log_{10}$ of the $CCID_{50}$.

With each of the assays having been carried out in duplicate, the following results table is obtained:

	Type 1	Type 2	Type 3	Type 4
Monovalent compositions:				
Assay 1	3.6	4.7	5.1	2.6
Assay 2	3.8	4.8	5.7	2.9
Tetravalent mixture:				
Assay 1	3.0	4.3	5.6	1.9
Assay 2	3.3	4.6	5.2	2.4

It is noted that the results which were obtained are in accordance with the expected results; the difference in titer which is observed for each type of virus in the tetravalent mixture varies by about 0.5 $\log_{10}$ $CCID_{50}$, which corresponds to the 1/4 dilution to which each serotype is subjected when the mixture is prepared.

Thus, it is possible, in accordance with the invention, to use non-neutralizing monoclonal antibodies to assay each of the serotypes which is present in a viral composition, without inducing any interference between the different serotypes.

Claims

1. Method for determining the virus quantity of each of the virus types or virus species in a composition which contains different species or types of live virus, characterized in that it comprises the following steps:
   - propagating the viruses of each type or species on cells which are permissive for the viruses but which do not induce any viral interference,
   - assaying each type or species of virus using a specific monoclonal antibody.

2. Method according to Claim 1, characterized in that the propagation is effected on Vero cells.

3. Method according to one of the preceding claims, characterized in that the composition comprising different species or types of live virus is a vaccine composition.

4. Method according to one of the preceding claims, characterized in that the composition comprising different species or types of live virus is a composition which comprises four serotypes of live attenuated dengue virus.

5. Method according to one of Claims 1 to 3, characterized in that the composition comprising different species or types of live virus is a composition which comprises three serotypes of live attenuated polio virus.

6. Method according to one of Claims 1 to 3, characterized in that the composition comprising different species or types of live virus is a composition which comprises different types of rotavirus.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NUMBER 020755-016

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITRATION METHOD FOR A COMPLEX VIRAL COMPOSITION

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Number _____

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/FR98/00678

on 03 April 1998

and was amended

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
FR	97/04371	03 April 1998	X Yes __ No
			__ Yes __ No
			__ Yes __ No
			__ Yes __ No
			__ Yes __ No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ _____
(Application Number) (Filing Date)

_____ _____
(Application Number) (Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NO. 020755-016

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	R. Danny Huntington	27,903	Gerald F. Swiss	30,113
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Platon N. Mandros	22,124	James W. Peterson	26,057	Charles F. Wieland III	33,096
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Address all telephone calls to: Norman H. Stepno at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NO. 020755-016

FULL NAME OF SOLE OR FIRST INVENTOR	SIGNATURE	DATE
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POST OFFICE ADDRESS		
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RESIDENCE		CITIZENSHIP
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RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		